

## 25 Spectrum of CFTR mutations in northern Portugal patients with CF and related phenotypes

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More than 1500 sequence variations have been reported in the CFTR gene, often with frequency variations related to geographic or ethnic origin, and which are found in both CF and related phenotypes – CFTR-related disorders (RD). To completely characterize the spectrum of CFTR mutations in northern Portugal we screened 26 paediatric CF patients as well as 54 patients with a CFTR-RD (46 with CBAVD and 8 with disseminated bronchiectasis). The study was carried out by an initial screening for 33 known CF mutations using a commercial kit, followed by an extensive screening by DGGE and sequencing analysis. In paediatric patients, the initial study showed a frequency of 36.5% for the F508del. The frequencies of the other 9 CF mutations were 3.8% for G542X, N1303K and R334W, while the other 5 were found only once (1.9%). This mutation analysis enabled the detection of 61.5% CF alleles. In the remaining uncharacterized CF alleles (38.5%), whole gene screening detected 3 further different mutations, increasing the mutation detection rate to 67.3%. In the 54 CFTR-RD patients, the initial analysis revealed that the F508del was found in 23.1% of the chromosomes whereas the IVS8(T)5 accounted for 26.9%. The frequencies of the other 6 CF mutations accounted for a further 17.4%. Extensive analysis detected 20 further mutations, increasing the mutation detection rate to 89.8%. Our results demonstrated a high allelic heterogeneity for CF in north of Portugal, as is reported for other southern European populations. Although an exhaustive screening strategy is being performed in a larger group of CF patients, the data presented here may be useful for improving genetic testing in Portugal as well as to genetic counselling.

## 27 The spectrum of CFTR mutations in populations from Romania

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**Objectives:** In Romania, beside the main Romanian population (89%) there are two other important ethnic groups – Hungarians (6.6%) and Gypsies (3%). The aim of this study was to estimate the frequency of CFTR mutations in Romanian population and in the two other ethnic groups.

**Methods:** Based on clinical findings and sweat test, we performed genetic testing for 29 CFTR mutations by ARMS-PCR in 63 patients (52 Romanians, 4 Hungarians and 7 Gypsies). 105 patients (91 Romanians, 9 Hungarians and 5 Gypsies) were previously investigated by other methods, PCR-SSCP, RFLP, direct sequencing.

**Results:** We identified 22 CFTR mutations (ΔF508, G542X, N1303K, 621+1G>T, W1282X, I148T, 2183 AA>G, G85E, 1898+1G>A, G576X, R553X, CFTRdel2,3(21kb), G817V, 3272–26A>G, 457TAT>G, 3849+10kbC>T, R1162X, 3849G>A, S1235R, R117H), four polymorphic variants – 2694T/G, 2694T/C, IVS8–5T, IVS8–7T and 2 new CFTR mutations – R735K and 1717–2A>G.

**Conclusions:** The most frequent mutation in Romanian population is ΔF508 (149 alleles, 52.1%), also for the Hungarian patients (16 alleles, 61.5%) and gypsy patients (15 alleles, 62.5%). However, the second most frequent mutation in Romanian population, G542X (11 alleles, 3.84%) was not found in Hungarian patients and one allele (4.16%) was G542X positive in gypsy patients. Beside ΔF508, we identified three more rare mutations in Hungarian patients (W1282X, R553X and R1162X) and no other mutations in gypsy patients. It is required to increase the number of patients with genetic diagnosis from the two ethnic groups (Hungarians and Gypsies) in order to obtain an improved estimate of their genetic structure.

## 26 The variety of Cystic Fibrosis genotypes of patients from Western Ukraine: ethnographical and population impact

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CFTR gene mutations vary in their frequency and distribution in different populations. Some CFTR mutations have a higher frequency in certain populations due to founder effect in religious, ethnic, geographical isolates.

Western region of Ukraine (10 millions inhabitants) is located at the strategic position of central Europe and was populated with Ukrainians, Poles, Jews, Russians, Germans, Armenians, Czechs, Slovaks, Hungarians, Gypsies. The region's historically dominant Ukrainian population (94.8%) diversifies demographically by the historical origin.

132 CF probands were detected. Eighteen different CFTR gene mutations were identified among them. Twenty two different genotypes (two mutations identified) are detected among studied group of patients.

43 (33%) CF patients have the same mutations in two alleles. Compound heterozygous F508del with other identified or unidentified (13.6%) CFTR mutation were detected at 45% of CF probands. 13 (9.8%) patients, all Ukrainians by nationality, have F508del/2184insA genotype. Ten of sixteen patients with 2184insA mutation (two homozygous among them) are from Lviv region.

N1303K mutation has been detected in combination with different CFTR mutations (F508del – 7 cases; 3849+10kbC>T, G542X, 2183AA>G, CFTRdele2,3 – each one case) as well one homozygous at thirteen CF patients, which mostly are from Volyn and Rivne region.

Slavic deletion CFTRdele2,3(21kb) were detected at ten patients ethnically Ukrainians and Russian.

Rare genotypes: 3272–11A>G homozygous, mutation 2721del11 and 1218 T>A (described for the first time) were identified in patients from Carpathian Mountains part of Lviv and Ivano-Frankivsk oblast, where geographical isolates are characteristic.

## 28 Molecular basis of cystic fibrosis in Republic of Macedonia: An update

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The molecular characterization of cystic fibrosis (CF) in Republic of Macedonia was initiated in 1989/90. The first ΔF508 screening showed lower frequency of the mutation than in any of the neighboring countries. With the development of a specialized CF centre at the University Pediatric Clinic in Skopje from 1997, the more reliable clinical criteria for diagnosis of CF were introduced. We performed re-evaluation of the clinical data of all patients diagnosed as having CF and the cases with non-conclusive clinical findings and borderline sweat test results were excluded from the CF registry. The aim of study was to update the molecular basis of CF in the Republic of Macedonia.

**Methods:** CFTR mutation analysis were performed in 115 unrelated patients with confirmed clinical diagnosis of CF. The molecular analysis included: detection of ΔF508 as the initial screening; denaturing gradient gel electrophoresis (DGGE) for mutation detection in exons: 11, 17b, 14b, 14a, 15, 20, 21, 9, 6a, 8, 5 and 18; and INNO-LiPA CFTR+17Tn kits for identification of CFTR mutations.

**Results:** The molecular defect was determinate in 89.1% (205/230) of the CF chromosomes. ΔF508 mutation was present in 73.9% (170/230). 12 other mutations, representing 15.2% of all CF alleles, were also identified. Only four of these were more common: G542X (5.6%), N1303K (3.1%), 621+G→T (1.7%) and CFTRdel21kb (1.3%). The other mutations, including three novel mutations (711+3A→G, 1811+1G→C and Y569C), were present in isolated cases.

**Conclusion:** The updated and extended data on the frequency and distribution of CF mutations detected in a cohort of 115 patients from the Republic of Macedonia showed high frequency of ΔF508 mutation. Only four additional mutations were found with a frequency of more than 1%.